bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Generation of FAM150A-conditioned and FAM150B-conditioned media

JG Jikui Guan

Updated date: Jan 5, 2021

An abbreviated version of this protocol was published in eLIFE in Sep 2015

FAM150A and FAM150B are activating ligands for anaplastic lymphoma kinase

DOI: 10.7554/eLife.09811

Detailed protocol

This protocol is used to generate FAM150A and FAM150B conditioned medium if you don't have purified ligand proteins. The plasmids used are pTT5-FAM150A-HA and pcDNA3-FAM150B-HA (both with HA tag at C terminal, for detailed sequence please see the end of this protocol).

1) Seed HEK293 cells (~5x10^6cell/dish) to 10-cm dishes in DMEM complete medium;
2) When the cells reach 80-90% confluence (monolayer), transfect with 8ug of pcDNA3 empty vector (control) or pTT5FAM150A-HA or pcDNA3-FAM150B-HA plasmid using Lipofectamine 2000 or Lipofectamine 3000 (less toxicity) according to manufacturer's recommendation;
3) Six hours after transfection, change the medium to fresh medium without serum;
4) Further culture the cells for another 48 hours and then collect the medium;
5) Clarify the medium by centrifugation at 2,000rpm for 5 minutes to remove cells and cell debris;
6) Keep medium at 4C fridge (can last for two weeks) or aliquote and freeze at -20C.

pTT5-FAM150A-HA (modified based on pTT5-FAM150A, a generous gift from Five Prime Therapeutics), the sequence of FAM150A-HA is shown below (Yellow: FAM150A CDS, green: linker, cyan: HA tag, red letters: start and stop codons)

ATGCGGCCCC TTAAGCCCGG CGCCCCTTTG CCCGCACTCT TCCTGCTGGC GCTGGCTTTG TCCCCGCACG GAGCCCACGG GAGGCCCCGG GGGCGCAGGG
GAGCGCGCGT CACGGATAAG GAGCCCAAGC CGTTGCTTTT CCTCCCCGCG GCCGGGGCCG GCCGGACTCC CAGCGGCTCC CGGAGCGCAG AAATATTCCC
AAGAGACTCT AACTTAAAAG ACAAATTCAT AAAGCATTTC ACAGGGCCGG TCACATTTTC ACCAGAATGC AGCAAACATT TCCACCGACT CTATTACAAT
ACCAGGGAGT GCTCAACGCC AGCTTATTAC AAAAGATGTG CTAGATTGTT AACAAGATTA GCAGTGAGTC CACTGTGCTC CCAGACCACG CGTTACCCAT
ACGATGTTCC AGATTACGCT GTT**TAA**

pcDNA3-FAM150B-HA was synthesized by the Palmer lab, the sequence of FAM150B-HA with flanked HindIII and NotI sites is shown below (green: restriction enzyme sites, yellow: FAM150B CDS, cyan: HA tag, red letters: start and stop codons)

AAGCTTCACC **ATG**CGCGGAC CCGGGCACCC CCTCCTCCTG GGGCTGCTGC TGGTGCTGGG GGCGGCGGGG CGCGGCCGGG GGGGCGCGGA GCCCCGGGAG
CCGGCGGACG GACAGGCGCT GCTGCGGCTG GTGGTGGAAC TCGTCCAGGA GCTGCGGAAG CACCACTCGG CGGAGCACAA GGGCCTGCAG CTCCTCGGGC
GGGACTGCGC CCTGGGCCGC GCGGAGGCGG CGGGGCTGGG GCCTTCGCCG GAGCAGCGAG TGGAAATTGT TCCTCGAGAT CTGAGGATGA AGGACAAGTT
TCTAAAACAC CTTACAGGCC CTCTTTATTT TAGTCCAAAG TGCAGCAAAC ACTTCCATAG ACTTTATCAC AACACCAGAG ACTGCACCAT
TCCTGCATAC TATAAAAGAT GCGCCAGGCT TCTTACCCGG CTGGCTGTCA GTCCAGTGTG CATGGAGGAT AAGCAGTACC CATACGATGT TCCAGATTAC
GCT**TAG**CGGC CGC

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Guan, J. (2021). Generation of FAM150A-conditioned and FAM150B-conditioned media. Bio-protocol Preprint. bio-protocol.org/prep732.
2. Guan, J., Umapathy, G., Yamazaki, Y., Wolfstetter, G., Mendoza, P., Pfeifer, K., Mohammed, A., Hugosson, F., Zhang, H., Hsu, A. W., Halenbeck, R., Hallberg, B. and Palmer, R. H.(2015). FAM150A and FAM150B are activating ligands for anaplastic lymphoma kinase. eLIFE. DOI: 10.7554/eLife.09811

Copyright: Content may be subjected to copyright.